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A PARADOXICAL ROLE FOR EICOSANOIDS: RADIOPROTECTANTS AND RADIOSENSITIZERS

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ABSTRACT

Understanding the radiobiology of eicosanoids is complicated by their ability to act as mediators of damage and recovery and as radioprotective agents. Changes in the tissue concentrations of eicosanoids following irradiation are dependent on several factors, including the type of eicosanoid, time postirradiation, radiation dose, and other contributing mediators and enzyme changes in the surrounding microenvironment. Many of these same prostaglandins and the leukotrienes have been shown to be radioprotective when given before irradiation. (Reprints)

INTRODUCTION

The eicosanoids are a group of biological mediators that have received attention in the field of radiobiology as mediators of radiation injury (1-3) and recently as radioprotective agents (4-6). They are metabolites of arachidonic acid (Figure 1), an essential 20-carbon fatty acid containing four unsaturated double bonds (7). Arachidonic acid is primarily esterified in the second position of the glycerol backbone of phospholipids in the cell membranes. Ultraviolet and ionizing radiation stimulate the release of free arachidonic acid through the action of phospholipases (8-10). Increased calcium concentrations also stimulate the phospholipase release of arachidonic acid. Following arachidonic acid release, one of three events occurs (outlined in Figure 1): (a) re-esterification of the free arachidonic acid back into the cell membrane, or (b) and (c) metabolism through the arachidonic acid cascade. Arachidonic acid is metabolized (Figure 1) through either the cyclooxygenase pathway (B), leading to the formation of prostaglandins, thromboxane, and prostacyclin, or the lipoxygenase pathway (C), leading to the formation of leukotrienes, lipoxins, and hydroxy fatty acids. These compounds have a number of important physiological roles in vasoregulation, smooth muscle regulation, electrolyte balance, and neuroregulation, as well as pathological roles in inflammation, fever, pain, and shock (reviewed in reference 7).

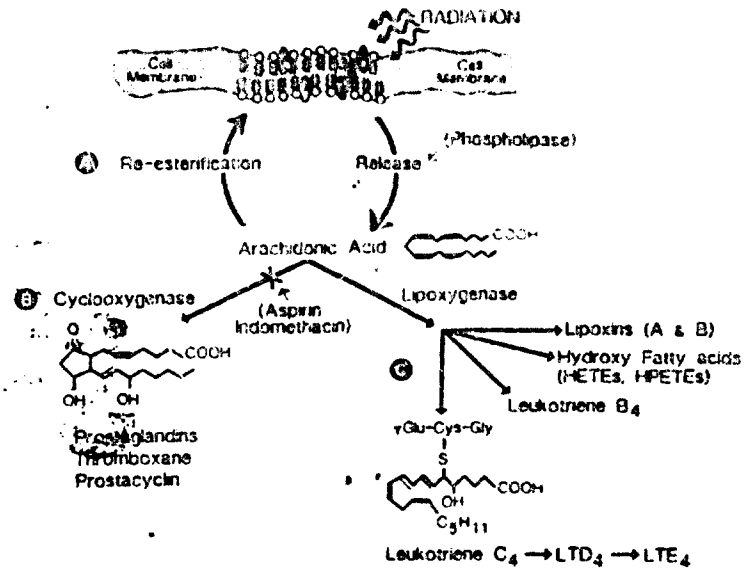

Fig. 1. Arachidonic acid cascade. Radiation stimulates release of free arachidonic acid from phospholipids of cell membrane. Once released, arachidonic acid may be (a) re-esterified back into cell membrane, (b) metabolized through cyclooxygenase pathway, or (c) metabolized through lipoxygenase pathway. Compounds that block either pathway may be exerting a beneficial effect by inhibiting production of a particular eicosanoid or by shunting more arachidonic acid into products of other pathway.

PROSTAGLANDINS IN RADIATION INJURY

The role of prostaglandins in radiation injury has been recently reviewed (11). Irradiation has been shown to alter tissue prostaglandin levels, producing both elevations (12-17) and decreases (1,17,18). The response following irradiation depends on dose received (18,19), tissue irradiated (1,13-19), and time of determination postirradiation (1). Some radiation-induced alterations in prostaglandin synthesis may persist for months after exposure to radiation or, conversely, may not become altered until several months postexposure (12). Prostaglandin changes following irradiation have been demonstrated *in vitro* in fibroblasts (11), endothelial cells (20), tissue slices, homogenates (21,22), and urine (23). Several effects of radiation on prostaglandin metabolism in the blood vessels are summarized in Figure 2. Alteration of tissue prostaglandin levels may result from either anabolic or catabolic processes, or from combinations of the two. Exposure of minipig skin to X radiation results in the increased production of prostaglandin E_2 (PGE_2) over the first 24 hours postirradiation (13). Early PGE_2 elevation in X-irradiated minipig skin is followed by a progressive decrease in PGE_2, which corresponds to an increase in prostaglandin F_2 (PGF_2) tissue levels. These latter processes are the result of an increase in 9-keto prostaglandin reductase, an enzyme that converts PGE_2 to PGF_2. There is an association between alterations in the spleen prostaglandin levels of

irradiated mice and alterations in the activity of prostaglandin dehydrogenase (24), an enzyme responsible for prostaglandin inactivation. Increases in prostaglandin E_1 (PGE_1) in the spleen postirradiation are caused by decreased enzymatic catabolism, leading to accumulation.

A role for prostaglandins as mediators of radiation injury has been suggested in experiments using cyclooxygenase-inhibiting drugs, such as indomethacin (3) or aspirin (2,25), to reduce specific radiation-induced inflammatory responses. These experiments indicate that prostaglandins contribute to radiation-induced ocular tissue inflammation in rabbits (2), mucositosis in humans (14), esophagitis in opossums (3), and gastrointestinal syndrome in mice (26). Prostaglandins may mediate inflammation through increased extravasation, vasoregulation, and fever and as chemotactic factors for phagocytic white blood cells (reviewed recently in references 7,27). Radiation may also affect the ability of the receptor to bind the prostaglandin and induce a specific function. The specific binding of prostaglandin E_2 is decreased in the spleens and small intestines of irradiated mice (28). The effects of indomethacin on alteration of whole animal survival are contradictory, and have been shown to either enhance survival (29) or have no effect (30). The reasons for the different responses to nonsteroidal anti-inflammatory drugs are not

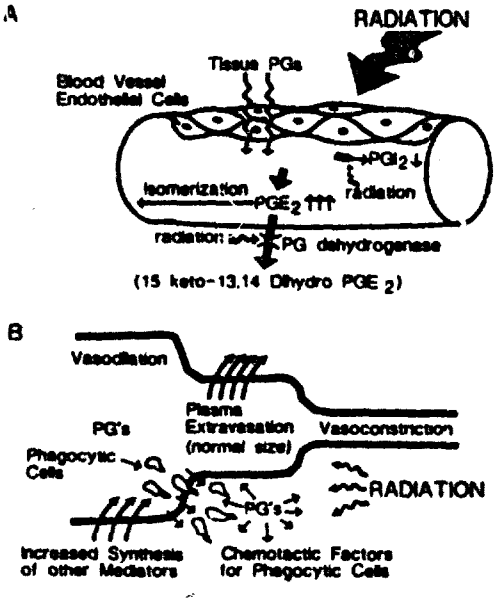

Fig. 2. A: Effects of radiation on prostaglandin metabolism by blood vessels. This figure presents a brief summary and is based on observations observed in human umbilical arteries (ref. 18) and in mouse spleen (ref. 24). B: Prostaglandin effects on blood vessels. Radiation induces an alteration of prostaglandin concentrations. These compounds may be either vasodilatory or vasoconstrictive, depending on class of prostaglandin released, particular vascular site, and prostaglandin concentration. As indicated, they may induce synthesis of other mediators or act as chemotactic agents.

known, but one may speculate that they are related to the dosage and schedule of administration.

PROSTAGLANDINS AND LEUKOTRIENES IN RADIOPROTECTION

Paradoxically, the prostaglandins have pathological roles in damage, but they also function as radioprotectants for cells in culture (6,31) and hematopoietic and intestinal stem cells (4) *in vivo*, and also enhance whole animal survival (5) Several studies on prostaglandin-induced modification of radiosensitivity in cell culture provide evidence that protection may be associated with elevations in cyclic AMP (6,31). These studies have centered on the use of PGE_1, a potent cAMP stimulus. For prostaglandin enhancement of whole animal survival from otherwise lethal exposure to ionizing radiation, the processes are more complicated, and the basic underlying mechanism(s) remains unknown. The most effective prostaglandin in terms of whole animal survival (31; Walden et al., submitted) is 16,16-dimethyl prostaglandin E_2 ($DiPGE_2$) (Figure 3), an analog of the naturally occurring prostaglandin E_2. Misoprostil, an analog of PGE_1, appears to be more effective for protection of the intestinal crypt cells (32). $DiPGE_2$ has a biological half-life in the tens of minutes (5), as illustrated and explained in Figure 3. The naturally occurring prostaglandin E_2 has a half-life of 2 minutes (7). Forty μg of $DiPGE_2$/mouse (1.6 mg/kg body weight) enhances the LD50/30 of mice exposed to cobalt-60 gamma radiation, providing a dose modification factor of 1.72 (5). Radioprotection by eicosanoids is both time and dose dependent, and must be

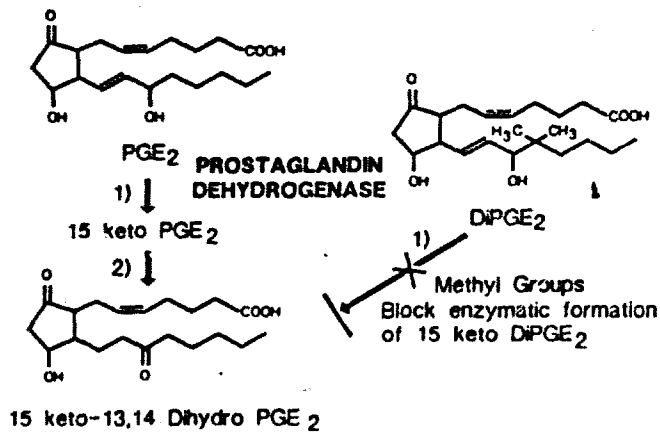

Fig. 3. Inactivation of prostaglandin E_2 but not 16,16-dimethyl prostaglandin E_2 ($DiPGE_2$). Prostaglandin E_2 in vasculature is normally inactivated by lung through action of prostaglandin dehydrogenase. Methyl groups in 16 position of $DiPGE_2$ prevent this inactivation step from occurring at 15 position, extending biological half-life. $DiPGE_2$ is primarily inactivated by liver (ref. 39).

administered just prior (approximately 15 min) to irradiation (5,33; Walden et al., submitted). There does not appear to be a common vascular end point induced by radioprotective eicosanoids, since some are vasodilatory and others are vasoconstrictive, and there is no general effect on the hematocrit (Walden et al., submitted). Pharmacological studies indicate that the $DiPGE_2$ protection results from the parent analog rather than a metabolite. However, the optimal period of radioprotection does not correlate with the optimal concentrations of $DiPGE_2$ in the tissue (5).

A decrease in locomotor activity (33) is produced by radioprotective concentrations of $DiPGE_2$. The optimal time for protection (5-60 min) is shorter than the duration of the locomotor behavior decrement (33). A marked depression of the locomotor activity occurred within 5 min postadministration of 10 μg $DiPGE_2$/mouse or greater. The behavior of $DiPGE_2$-treated mice returns to control levels by 6 hours following a 10-μg $DiPGE_2$ dose and 30 hours following a 40-μg dose (33). Diarrhea is another undesirable effect of some but not all of the radioprotective eicosanoids (Walden et al., submitted). It may be possible in the future to separate the detrimental effects without a decrease in protective efficacy.

Most work on the radiobiology of arachidonic acid metabolites to date has centered on the prostaglandins, primarily because the standards and radioimmunoassay kits for analyses are commercially available. Arachidonic acid may also be metabolized by the lipoxygenase pathway (Figure 1) to form leukotrienes and hydroxy fatty acids. Ultraviolet-B irradiation of human skin produced a threefold elevation of 12-hydroxy eicosatetraenoic acid 24 hours postirradiation, although no changes in leukotriene B_4 levels were observed (34). Leukotriene C_4, a thiol ether of glutathione and the triene-containing 20-carbon backbone, is elevated in the plasma of mice receiving hematoporphyrin derivative-induced phototherapy (400- to 410-nm wavelength radiation) and may be related to mast cell degranulation (35). Leukotriene C_4 modifies the radiosensitivity of V79A03 Chinese hamster cells in culture (36). Two-hour pretreatment with 2.5 μM leukotriene C_4 doubles the number of cells surviving a subsequent X irradiation (reproductive survival, based on colony formation). This protection is concentration dependent and appears to be associated with specific leukotriene C4-binding sites on the cell surface. Leukotriene C_4 also induces radioprotection and enhances the LD50/30 of mice (Walden, submitted).

Future research directions in eicosanoid radioprotection need to focus on the mechanism of action for this novel class of biological mediators/radioprotective agents. Effective radioprotective concentrations of arachidonic acid metabolites are in the μg/animal range (1.6 μg/kg body weight) (4,5), compared to mg/mouse (200-800 mg/kg body weight) required for the classical thiol radioprotectants. Several mechanisms may be involved in protection. The role of specific receptor activation provides the opportunity to enhance and control the protection. If a primary component of protection is receptor mediated, it may be possible to selectively protect all normal tissues that have receptors, but not those normal and tumor tissues that lack the receptor. Eicosanoid-induced events mediated on the cellular level by a receptor may enhance their effects on these cells and also other tissues

through systemic responses to eicosanoids that might occur through the cardiovascular system. Actions at these higher levels may play significant roles in the radioprotection. The success of the long-lived $DiPGE_2$, an analog of PGE_2, as a protective agent points to the need to develop other biologically stable eicosanoid analogs that retain radioprotective efficacy with minimal side effects.

Tumors secreting biological mediators with protective activity may modify the efficacy of the therapy. This appears to be the case for several of the prostaglandin-secreting tumors (37). The prostaglandin may conceivably protect the tumor at the cellular level by elevation of cyclic AMP (5,31) or glutathione (38), or at a systemic level by suppression of the immune system (1). An interesting set of experiments relating to this issue were conducted using the HSDM1C1 mouse fibrosarcoma cell line, which has been shown to secrete high levels of PGE_2 (38). Radiation clonogenic survival curves were performed in the presence or absence of flurbiprofen, a cyclooxygenase inhibitor. The two survival curves do not significantly differ, and may indicate that the PGE_2 produced by this tumor cell line does not feed back and modify its own cellular radiosensitivity. It is not known whether this cell line has receptors to PGE_2, and if the radioprotection is receptor mediated, then the lack of PGE_2 receptors may explain the inability of PGE_2 to act as a radioprotectant in this cell line.

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